ENHANCED PRODUCTION OF RECOMBINANT PROTEINS BY TRANSIENT TRANSFECTION OF SUSPENSION-GROWING MAMMALIAN CELLS

FIELD OF THE INVENTION

[0001] The invention relates to processes for producing recombinant proteins, in particular to a new process for an enhanced transient expression of a recombinant protein in host mammalian cells, and to new expression vectors, cell lines and culture media adapted to carrying out the process.

BACKGROUND OF THE INVENTION

[0002] Mammalian cells are an established expression system in the biotechnology industry for the production of recombinant proteins (r-proteins). In contrast to lower eukaryotes or prokaryotes, mammalian cells provide active r-proteins that possess relevant post-translational modifications. However, in order to obtain sufficient amount of protein for structure/activity analyses or high-throughput screenings, one needs to go through the long and tedious process of stable transfectoma isolation and characterization. As an alternative, the small-scale, transient transfection of mammalian cells grown in monolayers can generate significant amount of r-proteins (Cullen B. R., *Methods Enzymol.,* 152, 684-704 (1987); Blasey H. D. et al., *Cytotechnology,* 18, 183-192 (1996); Cachianes G. et al., *Biotechniques,* 15, 255-259 (1993)), but scalability of this process is limited by culture surface availability. The use of the well-established calcium phosphate precipitation technique or the recently described cationic polymer polyethylenimine (PEI) (Boussif O. et al., *Proc, Natl. Acad. Sci. USA,* 92, 7297-7301 (1995)) provides cost-effective ways of introducing plasmid DNA into mammalian cells. A major breakthrough has recently emerged for the fast production of milligram amounts of recombinant proteins when these gene transfer vehicles were shown to be effective for large-scale transient transfection of mammalian cells grown in suspension culture (Jordan M. et al., *Cytotechnology,* 26, 39-47 (1998); Schlaeger E-J. et al, *Cytotechnology,* 30, 71-83 (1999); Wurm F. et al., *Curr. Opin. Biotechnol.,* 10, 156-159 (1999)).

[0003] For an optimal large-scale transient transfection and r-protein expression in mammalian cells, four key aspects are to be taken into account, namely 1) the cell line, 2) the expression vector, 3) the transfection vehicle and 4) the culture medium. The human 293 cell line (a human embryonic kidney cell line containing the E1 region of human Ad5 adenovirus DNA) is widely used for r-protein production as it offers many advantages, such as high transfection yields with most common gene transfer vehicles, is easily grown in suspension culture, and can be adapted to serum-free media. Moreover, two genetic variants of the 293 cell line, the 293E and 293T cell lines, expressing the Epstein-Barr virus (EBV) Nuclear Antigen 1 (EBNA1) and the SV40 large-T antigen, respectively, allow episomal (extrachromosomal) amplification of plasmids containing the viral EBV (293E) or SV40 (293T) origins of replication. These cell lines are therefore expected to increase r-protein expression levels, by permitting more plasmid copies to persist in the transfected cells throughout the production phase (Van Craenenbroeck H. et al., *Eur. J. Biochem.,* 267, 5665-5678 (2000)).

[0004] The second important issue for high level r-protein expression is the use of vectors having promoters that are highly active in the host cell line, such as the human cytomegalovirus (CMV) promoter (Foecking M. K. et al, *Gene,* 45, 101-105 (1985)). This promoter is particularly powerful in 293 cells, where it has been shown to be strongly transactivated by the constitutively expressed adenovirus E1a protein (Gorman C. M. et al., *Virology,* 171, 377-385 (1989)). Moreover, a highly efficient expression cassette using this promoter has been recently described that provides adenovirus-mediated transgene expression levels reaching up to 20% of total cell proteins (TCP) (Massie B. et al., *J. Virol.,* 72, 2289-2296 (1998); Massie B. et al., *Cytotechnology,* 28, 53-64 (1998)).

[0005] The third aspect is related to gene transfer reagent efficacy. Even though many highly effective gene transfer reagents are commercially available, only few are cost-effective when considering operations at the multi-liters scale. For large-scale transient transfection applications, these reagents should also be simple to use, effective with suspension growing cells and have minimal cytotoxic effects. PEI satisfies most of these criteria, as it has high gene transfer activity in many cell lines while displaying low cytotoxicity (Boussif O., supra), is cost-effective, and efficiently transfects suspension growing 293 cells (Schlaeger E-J., supra). This polymer is available as both linear and branched isomers with a wide range of molecular weights and polydispersities, which physicochemical parameters are critical for efficient gene transfer activity (Godbey W. T. et al., *J. Control Release,* 60, 149-160 (1999).

[0006] The last key aspect for efficient r-protein expression by transient transfection relates to the culture medium. Some gene transfer reagents work only in serum-free media whereas others are less sensitive to the presence of serum. Also, as the presence of cellular by-products in conditioned medium is associated with poor transfection yield, it is often necessary to perform a complete medium change prior to transfection. However, this step does not satisfy the need for a robust large-scale transient transfection process.

[0007] Transient protein expression system are known in the prior art, for example the transient expression system disclosed in U.S. Pat. No. 5,024,939. However, these systems generally suffer from the above-discussed and other drawbacks and limitations and are not well suited to large-scale, high-throughput production of r-proteins. The present invention provides a transient expression system and process which is free of many such prior art limitations.

SUMMARY OF THE INVENTION

[0008] The invention provides a new process for the production of recombinant proteins, by transfection of suspension-growing eukaryotic cells with an expression vector comprising a first DNA sequence coding for the desired protein, said first DNA sequence being under control of a suitable promoter, and a second DNA sequence enhancing transcriptional activity of the promoter and increasing nuclear import of the expression vector. In a preferred embodiment, the second DNA sequence additionally supports an episomal replication of the vector in the transfected cells. The eukaryotic cells are preferably mammalian cells, more preferably the human embryonic kidney 293 cell line and its genetic variants, more preferably genetic variants